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The efficacy of novel NLRP3 inhibitors in a physiologically relevant *ex vivo* system using the organotypic brain slice inflammasome assay

Introduction

Inflammasomes are large intracellular protein complexes that assemble in response to danger signals and function as key components of the innate immune system. Different types of inflammasomes respond to different activating stimuli and once assembled, they activate Caspase-1, triggering the secretion of IL-1β and IL-18 and the induction of pyroptosis via cleavage of Gasdermin D (Figure 1). Inflammasome activation is essential for host defence, but its overactivation has been implicated in a variety of inflammatory diseases. The NLRP3 inflammasome responds to various damage- and pathogen-associated molecular patterns and is now an extensively validated therapeutic target for neurodegenerative diseases.

The Brain Slice Inflammasome Assay allows testing the efficacy of candidate drugs in a complex 3D *ex vivo* brain model, bridging the gap between *in vitro* cell-based assays and *in vivo* studies.

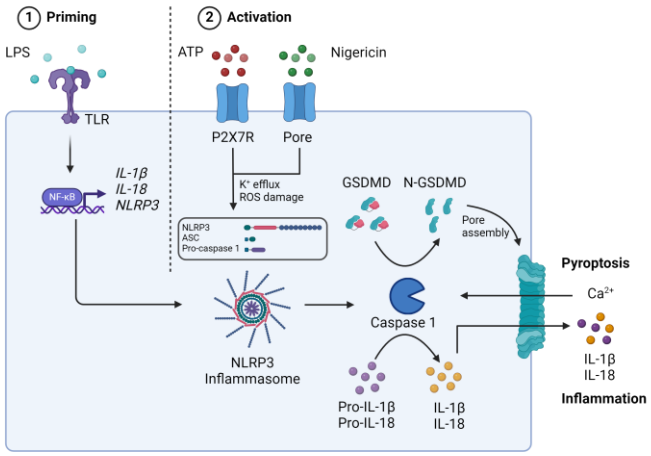

Figure 1. NLRP3 activation requires a priming stimulus and an activation stimulus. The priming stimulus activates NF-kB signalling, which in turn induces the precursor proteins of IL-1β and IL-18 as well as inflammasome component NLRP3, providing the elements for inflammasome complex formation and downstream signalling.

Both endogenous (e.g. ATP released from damaged cells) or exogenous (e.g. the ionophore Nigericin) signals can act as activating stimuli, triggering the cytosolic assembly of a complex comprising the sensor protein NLRP3, the adaptor protein ASC and the effector protein Caspase 1. The proteolytic activity of Caspase 1 leads to the generation of biologically active IL-1β and IL-18 as well as Gasdermin D. The latter forms pores in the plasma membrane, thus releasing IL-1β and IL-18 and triggering a specialised form of cell death known as pyroptosis.

Methods

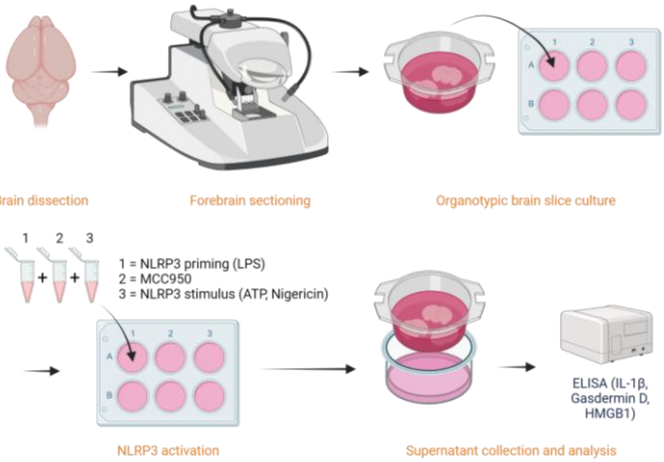

Figure 2. Coronal forebrain slices were generated from freshly dissected neonate C57BL/6 mouse brains using a vibratome and were placed onto tissue culture inserts in 6-well plates in culture medium. Each culture insert contained three slices collected from three different pups.

The slices were cultured for up to 14 days, after which they were pre-treated with either Vehicle or LPS, to induce NLRP3 priming. Next, the slices received a short pre-treatment with either vehicle or NLRP3 inhibitor MCC950, followed by addition of an NLRP3 activating stimulus (either ATP or Nigericin). Tissue culture supernatants were then collected and analysed by ELISA to detect secreted levels of IL-1β, Gasdermin D or HMGB1.

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Results

Selective activation of the NLRP3 inflammasome with ATP or Nigericin induces robust IL-1β secretion.

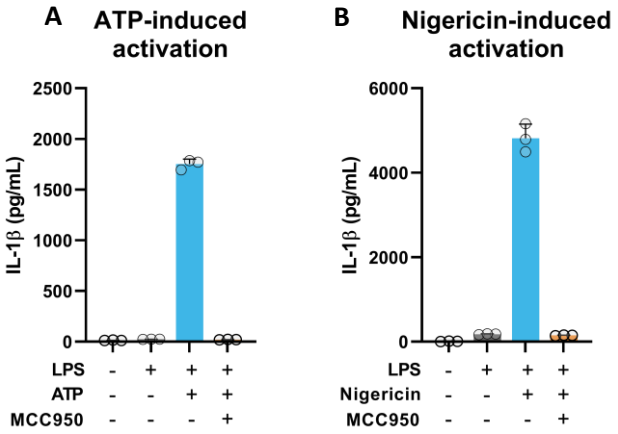

Figure 3. Secreted levels of IL-1β in tissue culture supernatants from slices treated with either LPS alone, LPS + ATP (A) or LPS + Nigericin (B), either in the absence or presence of NLRP3 inhibitor MCC950.

The secretion of multiple NLRP3 activation markers is concentration-dependently inhibited by MCC950.

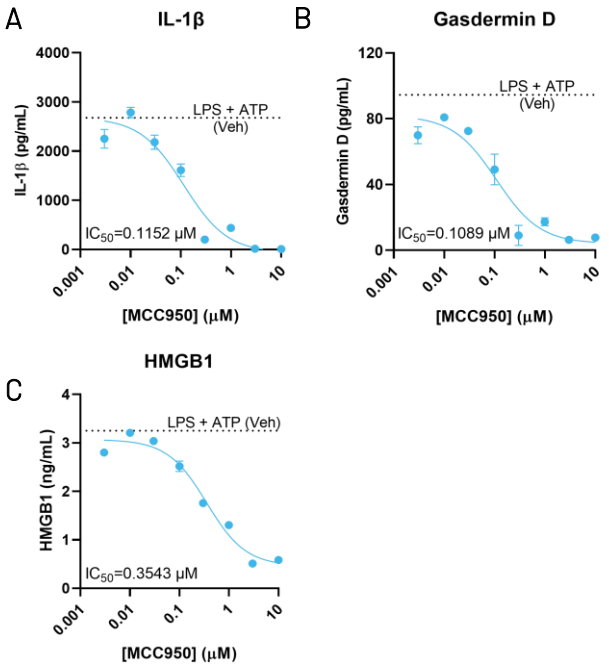

Figure 4. Secreted levels of IL-1β (A), Gasdermin D (B), HMGB1 (C) in tissue culture supernatants from slices treated with LPS + ATP, either in the absence or presence of increasing concentrations of NLRP3 inhibitor MC950.

After supernatant collection, brain slices can be utilised for various end-point readouts, including western blotting, immunohistochemistry, qPCR, and RNAseq.

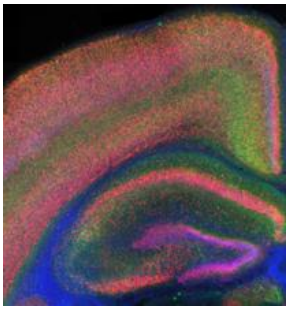

Figure 5. Wide field fluorescence image of a coronal organotypic brain slice stained with antibodies to Neurofilament heavy chain (green), NeuN (red), and counterstained with DAPI (blue). Image acquired with Vectra Polaris™ Imaging System.

Conclusions

NLRP3 inflammasome activation is not only a consequence of the CNS tissue damage that occurs in neurodegenerative diseases, but also a key driver of the neuroinflammatory processes underlying their pathogenesis. Therefore, CNS-directed NLRP3 inhibitors have the potential to treat a range of neurodegenerative diseases, including Alzheimer's and Parkinson's disease. Here we show that rodent organotypic brain slices accurately model NLRP3-induced CNS inflammatory responses and allow measuring the IC_{50} of candidate therapeutics targeting NLRP3 activation pathways in the CNS. This model enables testing drug candidates in a complex CNS environment, de-risking the transition to *in vivo* studies.

Key Features

- Preserved CNS cell populations and cytoarchitecture allow testing cellular responses in the native environment.
- Provides robust, reproducible readouts assessing NLRP3 activation and inhibition.
- Can be adapted to investigate drug effects on other inflammasomes.
- Compatible with multiple end-point readouts, allows in-depth MOA characterisation.
- Generates valuable CNS data quickly and cost-effectively compared to *in vivo* studies.

Frith Knoll Road, Chapel-en-le-Frith, Derbyshire, SK23 0PG, United Kingdom
www.conceptlifesciences.com • 01298 816700 • LinkedIn • Contact Us